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HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

September 28, 2001

MEMORANDUM

SUBJECT: EPA Review of "Dissipation of Dislodgeable Residues of Topsin® M from Strawberry Leaves" (MRID No. 448662-01), PC Code # 102001, DP Barcode D277643

FROM: Gary Bangs Barry E. O'Keefe for
Reregistration Branch 3
Health Effects Division (7509C)

TO: Deanna Scher, Chemical Review Manager
Special Review and Registration Division (7508C)

THROUGH: Steve Knizner, Branch Senior Scientist
Reregistration Branch 3
Health Effects Division (7509C)

This study partially met the requirement in OPPTS Series 875 of the Occupational and Residential Exposure Test Guidelines (U.S. EPA 1997) and can be useful in determining exposures to thiophanate-methyl residues on strawberry leaves or extrapolated to similar crops treated by ground-based spraying and adjusted for application rates. The California and North Carolina residues and dissipation patterns were sufficiently similar to be averaged together with good correlation and can be used for estimating dissipation of thiophanate-methyl on similar crops where rainfall is present. Residues of the breakdown product, MBC, were negligible.

EXECUTIVE SUMMARY

The study partially met the OPPTS 875 Guideline criteria. Dissipation of dislodgeable foliar residues of Topsin M (70 percent thiophanate-methyl) on strawberry leaves were quantified. Two foliar applications at the maximum label rate (actual rate 0.72 lb a.i. per acre, but less than the maximum *seasonal* application of 2.8 lbs a.i. per acre) were applied to *Seascape* strawberry plants in California and *Chandler* strawberry plants in North Carolina using groundboom equipment. The two applications were scheduled to be made 7 days apart at both sites; however, the second application at the NC site took place 8 days after the first application due to inclement weather. The author proposed that the use of this product on strawberry plants in California represented a reasonable worst-case scenario for potential exposure to individuals as per the exposure activity database compiled by the Agricultural Re-entry Task Force (ARTF). However, the California study had unusually high rainfall (total rainfall during study period was 1.5"). The California and North Carolina sites represent important climatic conditions and covers the significant climate variations. Drip or furrow irrigation was applied during the study when rainfall was insufficient at the two sites to maintain healthy, representative plants. These irrigation methods were typical for the respective areas and did not result in water contacting the foliar surfaces. Rain fell repeatedly during both test sites, and two days after the second application in North Carolina, and on the day of second application and the next day in California. Total rainfall during the study period at the North Carolina site was 10.4", 1.5" at the California site..

The thiophanate-methyl residue levels peaked immediately after both applications and then steadily declined to below the LOQ by Day 7 after the second application at the NC site. It rained the same day as the second application at the CA site and therefore, the residue recoveries were already below LOQ by Day 1 after the second application. The highest average DFR values were 1,212 µg (3.03 $\mu g/cm^2$) corrected thiophanate-methyl for NC and 962 μg (2.4 $\mu g/cm^2$) thiophanate-methyl for CA. Residues of MBC were negligible, with the highest average DFR equal to 30 μg (0.075 $\mu g/cm^2$) at the CA site and 26 μg (0.065 $\mu g/cm^2$) at the NC site. Laboratory and field fortification recoveries averaged over 90% at both sites, but the highest field fortification level had average recoveries less than 90% and the corresponding DFRs were corrected..

Overall, this study met most of the EPA OPPTS 875 test guidelines and will be used in the thiophanate-methyl risk assessment. Significant issues included:

- the maximum seasonal application rate of 2.8 lb ai/A was not applied;
- it could not be determined from the report if the collected field samples were dislodged within 4 hours after collection as specified in the field protocol;
- coefficients of variance for replicate samples at the NC site ranged from 5.4 percent to 44.6 percent for Thiophanate-methyl/MBC combined;
- OPPTS 875.2100 requires that DFR data be collected from at least three geographically distinct locations for each formulation, but DFR samples were collected from only two locations.

In spite of the deviations from Guidelines, the study is of sufficient quality to be used in the thiophanate-methyl risk assessment. The data sets were analyzed by the Agency using semi-log regression of the thiophanate-methyl and MBC residues for each site separately. Thiophanate-methyl and MBC should be considered separately due to different toxicity profiles. For the NC site, the regression analysis was run using Day 0 to Day 7 data after the second application and a dissipation half-life value of 1.4 days ($R^2 = 0.81$) was determined. For the CA site, the regression analysis was run using Day 0 to Day 7 data (pre-application #2) (excluding Day 1 data because all were non-detectable) after the first application (because of the rain event which occurred the day of the second application). The half-life calculated was 1.5 days ($R^2 = 0.75$) for the California site data. Therefore, it is appropriate to average the NC and CA site data for risk estimates. The predicted residues for the combined sites are shown in the table below. There is good correlation ($R^2 = 0.94$) of the log-transformed data, and the predicted values also agree well with the raw data at both sites. While many residue studies show regional or climatic variation in dissipation rates, the results of this study do not show significant differences between the two sites. Precipitation events may have affected the dissipation rates. Therefore, the averaged residues cannot be characterized as conservative, or high-end values.

MRID 448662-01 Dislodgeable Foliar Residues of Topsin M on Strawberries
Study Rate: 0.72 lb ai/acre
Slope: -0.430026
Intercept: 0.872957
R^2: 0.94

DAT (days)	Avg CA/NC DFR (ug/cm2) predicted
0	2.39
1	1.56
2	1.01
3	0.659
4	0.429
5	0.279
6	0.181
7	0.118
8	0.077
9	0.050
10	0.032
11	0.021
12	0.0137
13	8.9E-03
14	5.8E-03

Attachment:

Schaeffer, T., Anderson, S. Review of *Dissipation of Dislodgeable Residues of Topsin® M from Strawberry Leaves* (MRID No. 448662-01). Versar, Inc. August 31, 1999.

Versar inc.

MEMORANDUM

TO: Gary Bangs — cc: 3771.101, J. Becker, D. Baxter

FROM: Teri Schaeffer/Susan Anderson

DATE: August 31, 1999

SUBJECT: Review of *Dissipation of Dislodgeable Residues of Topsin® M from Strawberry Leaves* (MRID No. 448662-01)

This report reviews *Dissipation of Dislodgeable Residues of Topsin M from Strawberry Leaves,* submitted in support of the reregistration requirements for Topsin® M WSB. The requirements for this study are specified by the U.S. Environmental Protection Agency's (US-EPA) OPPTS Series 875, Occupational and Residential Exposure Test Guidelines, Group B: Postapplication Exposure Monitoring Test Guidelines, 875.2100, Transferable Residue Dissipation. The following information may be used to identify the study:

Title:	*Dissipation of Dislodgeable Residues of Topsin M from Strawberry Leaves,* Volume 2 of 2, 166 pages
Sponsor:	Richard S. Freedlander, Ph.D. Manager, Residue Chemistry Elf Atochem North America, Inc. 900 First Avenue King of Prussia, PA 19406 (610) 878-6477
Performing Laboratory: (Field Study) (PFI = Principal Field Investigator)	**(Site A)**: Grayson Research, LLC 1040 Grayson Farm Road Creedmoor, NC 27522 William P. Barney (PFI) (919)528-4925 **(Site B)**: Research for Hire, 1696 South Leggett Street Porterville, CA 93257 L. Scott Scheufele (PFI)
Analytical Laboratory:	Elf Atochem Residue Chemistry Department Laboratories 900 First Avenue King of Prussia, PA 19406
Author & Study Director:	Luis Castro Elf Atochem North America 900 First Avenue King of Prussia, PA 19406 (610)878-6471
Report Date:	June 28, 1999
Identifying Codes:	MRID # 448662-01, Elf Atochem Study Number KP-98-08

Executive Summary

This report reviews a study submitted by Elf Atochem North America, Inc. The purpose of the study was to characterize the dissipation of dislodgeable foliar residues (DFR) of thiophanate-methyl (TM) and methyl benzimidazole carbamate (MBC), which is a degradate of thiophanate-methyl, from strawberry plants grown in California and North Carolina. Topsin® M WSB is a fungicide containing 70 percent thiophanate-methyl as the active ingredient (a.i.) in a water soluble bag formulation.

Two foliar applications of 0.7 lb a.i./A each were applied to *Seascape* strawberry plants in California (4 miles SW of Porterville) and *Chandler* strawberry plants in North Carolina (Person County) using groundboom equipment. The two applications were scheduled to be made 7 days apart at both sites; however, the second application at the NC site took place 8 days after the first application due to inclement weather.

The TM residue levels peaked immediately after both applications and then steadily declined to below the LOQ by Day 7 after the second application at the NC site. It rained the same day as the second application at the CA site and therefore, the residue recoveries were already below LOQ by Day 1 after the second application. The highest average DFR values were 1,212 µg (3.03 $\mu g/cm^2$) corrected TM for NC and 962 μg (2.4 $\mu g/cm^2$) TM for CA. Residues of MBC were negligible, with the highest average DFR equal to 30 μg (0.075 $\mu g/cm^2$) at the CA site and 26 μg (0.065 $\mu g/cm^2$) at the NC site.

The author plotted the average TM (active ingredient) residue levels at each sampling interval in their statistical analysis. Data following the first application at the CA site and data following the second application at the NC site were plotted; due to inclement weather at the CA site. According to the author, the data fit a pseudo-first order linear regression analysis. A dissipation half-life of 0.94 days was calculated using TM DFR data from the second application at the NC site out to Day 5. A dissipation half-life of 1.53 days was calculated using TM DFR data from the first application out to Day 7 (pre-app. #2) at the CA site.

Versar re-analyzed the data sets using the Microsoft EXCEL 97® linear regression function, on the corrected combined TM and MBC residues for each site separately, and after averaging the results from the two sites together. For the NC site, the regression analysis was run using Day 0 to Day 5 data after the second application and a dissipation half-life value of 1.08 days (R^2 = 0.89) was determined. For the CA site, the regression analysis was run using Day 0 to Day 6 data (pre-application #2) after the first application (because of the rain event which occurred the day of the second application). A dissipation half-life of 1.78 days (R^2 = 0.76) was calculated. Versar ran an additional regression analysis after averaging data from both the NC site (data after the second application) and the CA site (data after the first application). This yielded a dissipation half-life of 1.42 days (R^2 = 0.90).

Overall, this study met most of the EPA OPPTS 875 test guidelines. Versar identified the following non-compliance and other issues:

- The maximum application rate was used (0.7 lb a.i./A) for two applications; however, the maximum seasonal application of 2.8 lb ai/A was not applied. Four applications would be necessary to reach the maximum seasonal application rate.

- The terms "limit of detection" (LOD) and "limit of quantitation" (LOQ) were used synonymously throughout this study. Therefore, there is uncertainty as to what the LOD and LOQ actually were.

- The analytical method did not appear to have been validated prior to initiation of the study or initiation of field sample analysis.

- The protocol stated that the collected field samples were to be dislodged within 4 hours after collection but whether this actually occurred could not be confirmed in the field phase summary.

- The reproducibility of replicate samples collected at the same time interval was poor. The coefficient of variance for replicate samples at the NC site ranged from 5.4 percent to 44.6 percent for TM/MBC combined.

- OPPTS 875.2100 (an Update to Subdivision K) generally requires that DFR data be collected from at least three geographically distinct locations for each formulation. In this study, DFR samples were collected from only two locations.

- On page 15 of the Study Report (5.1 Data used for kinetics calculations), the author reported that "only the data following the first application at the NC site and data following the second application at the CA site were used." The sites referred to should be reversed in this statement. The rain event which caused the problems occurred on the day of the second application at the CA site and not at the NC site. The author also stated that the data from the first day of sampling after the second application was anomalous at the CA site but it was data from the first day of sampling after the first application that was anomalous.

STUDY REVIEW

Study Background

This study was submitted in response to a Data Call-in Notice issued by EPA on October 18, 1995. The purpose of the study was to characterize the dissipation of foliar residues of thiophanate-methyl (CAS No. 23564-05-8) and methyl benzimidazole carbamate (CAS No. 17804-35-2), which is a degradate of thiophanate-methyl, from strawberry plants grown in California and North Carolina. The data are intended to assist in determination of worker re-entry intervals.

Topsin® M WSB is a fungicide containing 70 percent thiophanate-methyl as the active ingredient (a.i.) in a water soluble bag formulation. It is labeled for use to control a variety of fungal diseases in a number of commercially important pome fruit, stone fruit, berry, nut, cucurbit, legume and tuber crops.

The field portions of the study were directed by Grayson Research, LLC (Site A - North Carolina) and Research for Hire (Site B - California). Samples were collected between April 02, 1998 and July 27, 1998. Analysis of the samples was performed at the Elf Atochem Residue Chemistry Department Laboratories between May 6, 1998 and February 4, 1999.

Test Plot

Thiophanate-methyl was applied at two test sites. One was located in California (4 miles SW of Porterville) and the other in North Carolina (Person County). According to 1998 United States Department of Agriculture statistics, 80 percent of strawberries grown in the United States are grown in California and 2 percent are produced in North Carolina. The author proposed that the use of this product on strawberry plants in California represented a reasonable worst-case scenario for potential exposure to individuals as per the exposure activity database compiled by the Agricultural Re-entry Task Force (ARTF). The California and North Carolina sites represent important climatic conditions and covers the significant climate variations.

Each test site was subdivided into a treated plot and a control plot. The untreated plot at the NC site measured 30 feet by 20 feet and consisted of six 20-foot, double-planted beds. This untreated control plot was located 100 feet upwind and up-slope of the treated plot. The untreated plot at the CA site measured 10 feet by 100 feet and consisted of four 100-foot, double-planted beds. This untreated control plot was located at least 1000 feet upwind and up-slope of the treated plot. The treated plot at the NC site was 30 feet by 60 feet and consisted of six 60-foot, double planted beds. The treated plot was partitioned into three subplots each containing six double-planted rows measuring 20 feet in length. The treated plot at the CA site measured 20 feet by 200 feet and consisted of eight 200-foot, double-planted beds. This plot was partitioned into three replicate subplots (A, B, and C) which were 8 beds by 66 feet, 8 beds by 66 feet and 8 beds by 68 feet, respectively. Plot layouts were diagramed for all the treated and untreated plots and these were provided in the Study Report (pages 96 and 98).

In North Carolina, the strawberries (variety *Chandler*) were planted on September 26 and 27, 1997. In California, the strawberries (variety *Seascape*) were planted on March 6, 1997. The strawberry plants were maintained according to normal cultural practices. Drip or furrow irrigation was applied during the study when rainfall was insufficient at the two sites to maintain healthy, representative plants. These irrigation methods were typical for the respective areas and did not result in water contacting the foliar surfaces.

Maintenance chemicals were applied to both sites prior to the initiation of the studies. For the North Carolina site, one application of a fertilizer and urea was applied to the plot in 1997 prior to the planting of the strawberry plants and two applications of a herbicide (i.e., Poast) were applied in February of 1998 to the strawberry plants. The test product was applied twice in April of 1998. For the California site, three applications of fertilizer and three pesticide applications (two Brigade® WSB applications and one Devrinol® 50W) were made in the spring/summer of 1997. The test product was applied in the spring of 1998 and followed by two pesticide applications a little more than a month after the last test product application (see Table 1 for dates and amount applied).

A pesticide use history for both sites (dating back to 1995) was provided on pages 84 and 85 of the Study Report. The North Carolina site was fallow up until the time of planting. The California site produced various crops over the three years prior to the initiation of the study (e.g., tomato, lettuce, bell pepper, cucumber and strawberry). Head lettuce and cucumbers were also grown in the same area as the California untreated control plot. Lannate 50W (methyl parathion), Rovral 50 WP (iprodione), Dibrom 8E (naled), Danitol (fenpropathrin) and Diazinon 4E were all applied the previous year (1997) on the two crops in the control plot.

Table 1. Crop Maintenance & Topsin® M WSB Applications

Date	Maintenance Chemicals	Amount/Acre
	North Carolina Site	
July 1997	Sulfur coated urea (37-0-0)	350 lbs
July 1997	10-10-10 fertilizer	400 lbs
February 6&7 1998	Poast herbicide (sethoxydim)	1.5 pts
April 3, 1998	Topsin® M WSB (thiophanate-methyl)	0.72
April 11, 1998	Topsin® M WSB (thiophanate-methyl)	0.72
	California Site	
April 1997	20-20-20 fertilizer	14 lbs
April 1997	Devrinol 50 W (napropamide)	1.0 lbs a.i.
May 1997	Brigade WSB (bifenthrin)	0.3 lbs a.i.
May 1997	20-20-20 fertilizer	20 lbs
June 1997	Brigade WSB (bifentrhin)	0.2 lbs a.i.
June 1997	20-20-20 fertilizer	10 lbs
April 27, 1998	Topsin ® M WSB (thiophanate-methyl)	0.7 lbs
May 4, 1998	Topsin ® M WSB (thiophanate-methyl)	0.69 lbs
June 11, 1998	Kelthane (dicofol)	1.0 lbs a.i.
June 17, 1998	Agrimet (phorate)	0.06 lbs a.i.

Meteorology

Rainfall data for each study were collected from rain gauges located at the test plots. Minimum and maximum air and soil temperature, average humidity, average and maximum wind speed, wind direction, and total solar radiation data for the duration of the application and sample collection period in North Carolina were obtained from the North Carolina Agricultural Research Service, Tobacco Research Station in Oxford, North Carolina, approximately 16 miles southeast of the test plot. Minimum and maximum air temperature data for the duration of the application and sample collection period in California were obtained from NOAA Station #04 7077 5 at Daybell Nursery, Porterville, California approximately 4.5 miles northeast of the test plot. Relative humidity, soil temperature, mean wind speed, and wind direction were also recorded for the California site on the days of the test substance application but the source of this data was not reported.

Rain fell the day after the first application at the North Carolina site. The applications of the test substance were supposed to be 7 days apart but it rained on the seventh day at the North Carolina site and therefore the second application took place on the eighth day. Rain fell two days after the second application. All together, there were 26 rain events recorded from the day after the first application (April 4, 1998) through June 30, 1998 (i.e., 10.4 inches total). The final sample was collected on July 3, 1998 (see page 92 in the Study Report). Because of inclement weather, the sample collection events scheduled for Days 56 and 84 after the second application, occurred on Days 57 and 83, respectively.

Rain fell on the sixth day after the first application at the California site. The second application was made on the seventh day after the first application. Rain fell on the day of the second application as well as the day after the application. There were a total of seven rain events reported from May 3, 1998, through July 21, 1998 (i.e., 1.47 inches total). The final sample was collected on July 27, 1998. (See page 93 in the Study Report.)

Historical weather data on the minimum/maximum air temperatures and rainfall for the North Carolina site were obtained from NOAA Station # 7516, Roxboro, North Carolina, approximately 4 miles southeast of the test plot. The historical data dated back to 1961. (See page 90 in the Study Report.)

Historical weather data on the minimum/maximum air temperatures and rainfall for the California site were obtained from NOAA Station #04 7077 5 at Daybell Nursery, Porterville, California, approximately 4.5 miles northeast of the test site. This historical data dated back to 1988. Rainfall for the month of May 1998 (i.e., after the second application) measured 1.26 inches, while the average precipitation for May for the 10 years previous was 0.36 inches.

Materials and Applications

The Topsin® M WSB product label was provided in the Study Report (see pages 56-61). The label specifies a minimum re-application interval for Topsin® M WSB of 7 days. Two applications of Topsin® M WSB were made 8 days apart on April 3 and April 11, 1998 at the North Carolina site and 7 days apart on April 27 and May 4, 1998 at the California site. The maximum application rate used for this study was achieved by applying 0.7 lbs a.i. per acre during the first application followed by a second application of 0.7 lbs a.i./A, seven to eight days later (total of 1.4 lbs a.i. per season). The maximum application rate per application used in this study met label recommendations; however, the maximum application rate per season applied was only half of the label maximum seasonal application rate of 2.8 lb a.i. per season.

The application volume was 30 gallons of finished spray/acre. [A minimum application volume per acre for strawberries was not recommended on the product label.]

Each application at the North Carolina site was made with a tractor mounted broadcast boom sprayer, pressurized by a PTO driven diaphragm pump, and equipped with continuous bypass agitation of the spray solution. Ten TeeJet 8002 flat fan nozzles on 18 inch spacing were positioned approximately 18 inches above the foliar surface. It took two passes to cover the 30 foot wide plot. The actual application rates for both applications were 0.72 lb a.i. per acre.

Each application at the California site was made with a tractor mounted broadcast boom sprayer, pressurized by a PTO driven pump, and equipped with continuous bypass agitation of the spray solution. Twelve TeeJet D-4 hollow cone nozzles on 20 inch spacing were positioned approximately 18 inches above the foliar surface. One pass was sufficient enough to cover the 20 foot wide plot. The actual application rates was verified at 0.7 lb a.i. per acre for the first application and 0.69 lb a.i. per acre for the second application.

Application verification calculations were provided in Appendix C (pages 108-110) of the Study Report.

Sampling/ Residue Dislodging

Leaf punch samples were collected at the following intervals: just prior to each application, just after each application (after the spray had dried), on Days 1, 3, and 5 after the first application, and on Days 1, 3, 5, 7, 14, 21, 28, 42, 56, 70, and 84 after the second (final) application. At each interval, three replicate samples were collected from the treated plot (one from each of the three treated sub-plots). Seven samples were collected from the control plot at five designated sampling intervals; one was used as a control sample and the remaining six were used as field fortified samples. These samples were collected just after the first and second application, and on Days 14, 28 and 84 after the second application. All leaf punches were collected using a Birkestrand (or equivalent) leaf punch apparatus. Each composite sample consisted of 40 leaf punches, each approximately 2.5 cm in diameter, collected from several locations within each plot. Samples were collected randomly from within each plot area, avoiding border rows and plot ends. Two figures were provided (pages 96 and 98 in the Study Report) to diagram sampling areas within the plots. The total surface area (front and back) of a composite sample was 405 cm^2.

The study protocol stated that the foliar residues were to be dislodged within 4 hours of collection but the field phase summary did not state whether or not the samples were in fact dislodged within 4 hours of collection. Foliar residues were dislodged twice with 100 ml of an aqueous solution of 0.01 percent Aerosol® OT surfactant (American Cyanamid). Each 100 ml aliquot was added to the sample jar containing the leaf punches and then mechanically shaken for 10 minutes for each extraction. The aqueous extracts from both extractions were decanted and combined in a clean pre-labeled container to which 150 mg (+/- 10 mg) of L-cysteine had previously been added. The samples were then capped and placed in freezers.

QA/QC

Sample Handling & Storage

Leaf punch samples were stored in field coolers with substitute ice for transport to an appropriate site for dislodging. Separate coolers were used to field store and transport treated and untreated leaf discs. Field storage and transport temperatures ranged from 14 to 72 ° F at the NC trial, and 12 to 102 ° F at the CA trial. The leaf discs did not freeze at either site during field transport and storage. Leaf punch extracts (including field fortified samples) were stored at or below freezing at each field site until shipped frozen by ACDS freezer truck service. Untreated and treated samples were packed in separate shipping containers. All samples were received frozen and in good condition at the Elf Atochem laboratory. According to the protocol, a sample chain of custody was to be kept but this was never mentioned in either the field or analytical phase summaries. The sample storage conditions once the sample extracts reached the analytical laboratory were never discussed.

Sample History

Table 1c (see page 19) of the Study Report summarizes the sample handling history. This table was not sample ID specific. It only showed that there were three shipments of samples to the laboratory and that the longest storage interval was 107 days. Sample storage interval dates and analysis dates for each individual sample extract were not provided. A formal sample chain-of-custody was mentioned in the study protocol but was not provided for review nor discussed in the Study Report.

Analytical Methodology

All samples were analyzed by Elf Atochem's Residue Laboratories according to proprietary methods (see Appendix D of the Study Report). It was not clear whether the methods used were validated prior to conduction of the study or concurrently with the DFR sample analyses. Two different analytical methods were used for the analysis of the field samples. A more sensitive method was used for analysis of the low-level field fortifications. The reliability of the analytical methods was demonstrated by spiking untreated control samples with known amounts of thiophanate-methyl (TM) and methyl benzimidazole carbamate (MBC), and carrying these fortified samples through the method along with each set of DFR samples. The Study Report author stated that due to the acceptable recovery of the lab fortified samples and the absence of apparent residues of TM or MBC in the field controls, the method used was considered to have good accountability of the residues in the detergent washes and appropriate specificity for the DFR residues.

Briefly, for the regular method, the leaf extracts were brought to room temperature and shaken to mix thoroughly. A 2 mL aliquot was filtered through a 0.45 µm filter into two different autosampler vials. The residues were quantified using an HPLC. For the more sensitive method, the leaf extracts were brought to room temperature and shaken to mix thoroughly. A 25 mL aliquot was removed and a small amount of L-cysteine was added to the aliquot. An Oasis SPE cartridge was attached to a vacuum manifold and conditioned with successive washes of methanol and deionized water. The sample was percolated through the Oasis cartridge and the column was then washed with a mixture of methanol:water. The eluate and rinse was collected and evaporated to an approximate volume of 0.5 ml using a gentle stream of nitrogen. The concentrate was brought up to a final volume of 4.0 ml by adding a diluent containing 35 percent methanol and 65 percent water, and then the solution was buffered to a pH of 6.5. A small volume was then filtered through a 0.45 µm filter into two different autosampler vials. The residues were quantified using an HPLC.

The chromatographic conditions are provided on page 116 of the Study Report. Instrument linearity was verified each day samples were analyzed. According to the information presented in the Study Report, calibration curves were generated using six concentrations of the reference standards ranging from 0.05 to 2.0 µg/ml (0.025 µg/cm^2 to 1.0 µg/cm^2). Example chromatograms were included and showed good peak separation. However, the maximum DFR sample value was 2.7 µg/cm^2 (prior to data correction) and the maximum field fortified and lab fortified concentrations used were 5.0 µg/ml (2.5 µg/cm^2). No mention of dilutions was made and the raw data were not supplied. Therefore, the accuracy of these values could not be verified by the study reviewer.

Limits of Detection (LOD) & Limit of Quantitation (LOQ) and Control Samples

The LOD and the LOQ were not clearly distinguished and the terms seem to have been used synonymously. The more sensitive analytical method, requiring a concentration of the analytes, had a limit of quantitation (LOQ) of 0.008 µg/ml (0.004 µg/cm^2) for both TM and MBC. The "regular" method, which did not require the concentration step, had an LOQ of 0.05 µg/ml (0.025 µg/cm^2) for both TM and MBC.

At least one untreated control sample was included in each analytical set. Of 18 separate analyses of field control samples, only one positive result for TM was detected. This result (i.e., 13 µg TM) was barely above the LOQ of 10 µg (i.e., 0.025 µg/cm^2). This sample was reanalyzed and gave a non-detect result.

Laboratory Recovery

Laboratory fortification samples were analyzed concurrently with each set of samples by fortifying a control sample with an appropriate amount of TM and MBC. One fortification level was analyzed using the more "sensitive" method. Three control samples were fortified with 0.01 µg/ml of TM and MBC just prior to analysis. These samples yielded average recoveries of 97 percent TM ($\pm$ 2 percent) and 95 percent MBC ($\pm$ 7 percent). Only the low-level field fortification samples were analyzed using this method.

Additional laboratory recovery samples were fortified at levels ranging from 0.1 to 5.0 μg/ml. These were analyzed using the regular method (i.e., 14 samples). The average recoveries for TM and MBC were 91 percent ($\pm$ 9 percent) and 99 percent ($\pm$ 4 percent), respectively. The highest fortification concentration used did not exceed the levels found in the field samples. The highest fortification concentration was 5 μg/ml (2.5 $\mu g/cm^2$) while the highest field sample recovery was 2.7 $\mu g/cm^2$. Average recoveries are provided in Table 2, below.

Table 2. Average Laboratory Recoveries for TM and MBC

Spike Level (µg/ml)	n	TM Mean Recovery (%)	Standard Deviation* (%)	MBC Mean Recovery (%)	Standard Deviation* (%)
Sensitive Method					
0.01	3	96.7	± 2	95	± 7
Overall Average	3	97	± 2	95	± 7
Regular Method					
0.1	3	102	± 5	100.3	± 3
0.2	3	93	± 3	99	± 2
0.5	3	85.3	± 5	96.7	± 1
1	3	80.3	± 9	95.3	± 2
2	1	94	---	96	---
5	1	94	---	109	---
Overall Average	14	91	± 9	99	± 4

* When n<3 the standard deviation is not calculated.

Field Fortification Recovery

Seven replicate samples were taken from the control plots at five different sampling intervals and dislodged in the same fashion as the test samples. The field fortification samples were collected just after both applications and on Days 14, 28, and 84 after the second application. One of each set of these replicate samples was kept as a control sample. The remaining six samples in each set were prepared in triplicate at two fortification levels for each of the five different sampling intervals. The fortified samples were frozen, handled, and subjected to the same environmental conditions as the field samples. At the beginning of the study, the field fortifications for TM and MBC were prepared at 0.00625 $\mu g/cm^2$ and 0.0625 $\mu g/cm^2$. Due to the high residue levels found in the earliest sampling intervals following the applications, the fortification levels were increased to 0.500 $\mu g/cm^2$ and 2.50 $\mu g/cm^2$. However, the higher concentrated field fortification samples were prepared on Days 14 (NC), 28 (CA and NC) and 84 (CA and NC). Only one or two replicates were analyzed at this level because the treated field sample residues were below the LOQ by Day 14. Therefore, all samples collected after Day 21, except for one or two field fortified replicate samples, were not analyzed.

According to the author, the results of the analysis of these field fortification samples indicated that there was no gross loss of analyte during the storage and transport of the samples. The author reported the overall average recoveries for both sites combined were 95 percent (+ 17 percent) for TM and 94 percent (+ 11 percent) for MBC. The individual average recoveries for each fortification level at each site are summarized below in Table 3. [See also Tables 5a and 5b of the Study Report.]

Table 3. Average Field Fortification Recoveries for TM and MBC

TM				MBC			
µg/cm²	n	Mean Recovery (%)	Standard Deviation (%)	µg/cm²	n	Mean Recovery (%)	Standard Deviation (%)
North Carolina							
0.00625	9	94.7	± 13	0.00625	9	96.1	± 12
0.0625	9	102.8	± 28	0.0625	9	92.2	± 12
0.5	1	100	---	0.5	1	97	---
2.5	1	86	---	2.5	1	83	---
Overall Average	20	98	± 21	**Overall Average**	20	94	± 11
California							
0.00625	6	86.2	± 8	0.00625	6	97	± 10
0.0625	4	94	± 11	0.0625	4	98.5	± 2
0.5	2	93.5	---	0.5	2	83	---
2.5	1	92	---	2.5	1	66	---
Overall Average	13	91	± 9	**Overall Average**	13	93	± 12

Storage Stability Recovery

A separate storage stability study was not performed. The stability of TM in the dislodging solution (0.01 percent Aerosol® OT surfactant) was determined by the analysis of field fortified samples which were stored frozen with the field samples. The longest storage interval for a field sample before analysis was 107 days and 183 days for one of the field fortified spikes. The percent recovery for this field fortified sample was 102 percent for TM and 95 percent for MBC, both spiked at 0.0625 µg/cm^2. For additional field fortified sample analysis results, refer to the Field Fortification section above. The field fortification analysis results successfully demonstrated that TM/MBC residues were stable over the time period necessary to complete analysis of all study samples.

Results

Tables 4a and 4b summarize the combined foliar dislodgeable TM and MBC residue data for each sampling interval from the NC and CA test sites. The highest DFR residues were found to occur during the first sampling interval shortly after each application. The author noted that due to the excellent recoveries of TM and MBC, demonstrated by the field fortified samples, no correction was made to the treated sample data for any transportation or storage losses. This was taking into account the overall average recoveries for all concentrations combined. However, the average percent recoveries for the higher field fortified concentrations at the NC site were below 90 percent and Versar opted to correct the data points which corresponded to the higher field fortification levels. This affected the NC site data designated by * in Table 4a. The TM residue levels peaked immediately after both applications and then steadily declined to below the LOQ by Day 7 after the second application at the NC site. It rained the same day as the second application at the CA site and the residue levels were below LOQ by Day 1 after the second application. The highest average DFR values were 1,212 µg (3.03 µg/cm^2) corrected TM for NC and 962 µg (2.4 µg/cm^2) TM for CA. Residues of MBC were negligible with the highest average DFR equal to 30 µg (0.075 µg/cm^2) at the CA site and 26 µg (0.065 µg/cm^2) at the NC site. The greatest concentrations of MBC were evident in CA, but the author did not regress these data due to the low recoveries and the variability of the recoveries. Versar performed a regression analysis for the combined TM and MBC residues.

The author considered only TM (active ingredient) residues in the statistical analysis, and used the average of the three replicates taken at each sampling interval to calculate the dissipation kinetics. (See graphs on pages 32 through 39 in the Study Report). The data following the first application at the CA site and data following the second application at the NC site were plotted. The data from the first day of sampling after the first application at the CA site was anomalous. Neither TM nor MBC were detected in any of the three treated replicates. Reanalysis confirmed these results. These results were considered to be outliers because the samples from the previous sampling interval and the subsequent sampling intervals both contained considerable amounts of TM. These data were not included in the regression analysis. According to the author, the data fit a pseudo-first order linear regression analysis. A dissipation half-life of TM was calculated using data from the second application at the NC site out to Day 5. The result was a dissipation half-life of 0.94 days (an R^2 value was not provided). A dissipation half-life of TM was calculated using data from the first application out to Day 7 (pre-app. #2) at the CA site. The result was a dissipation half-life of 1.53 days (an R^2 value was not provided).

Table 4a. Combined TM and MBC DFRs After Two Applications of Topsin® M WSB at the North Carolina Site

Sampling Interval	TM + MBC ($\mu g/cm^2$)			
	Repli. 1	Repli. 2	Repli. 3	Avg.[a]
Pre-Application #1	<LOQ	<LOQ	<LOQ	0.025
Post-Application #1	2.75*	2.44*	2.79*	2.66*
Day 1 after App. #1	<LOQ	<LOQ	<LOQ	0.025
Day 3 after App. #1	<LOQ	<LOQ	<LOQ	0.025
Day 5 after App. #1	<LOQ	<LOQ	<LOQ	0.025
Pre-Application #2	<LOQ	<LOQ	<LOQ	0.025
Post-Application #2	3.08*	3.18*	2.86*	3.04*
Day 1 after App. #2	1.51	1.38	2.23*	1.71*
Day 3 after App. #2	0.20	0.19	0.26	0.22
Day 5 after App. #2	0.085	0.18	0.23	0.17
Day 7 after App. #2	<LOQ	<LOQ	<LOQ	0.025
Day 14 after App. #2	<LOQ	<LOQ	<LOQ	0.025
Day 21 after App. #2	<LOQ	<LOQ	<LOQ	0.025
Day 28 after App. #2	<LOQ	NA	NA	0.025
Day 42 after App. #2	NA	NA	NA	NA
Day 56 after App. #2	NA	NA	NA	NA
Day 70 after App. #2	NA	NA	NA	NA
Day 84 after App. #2	NA	NA	NA	NA

NA - Samples not analyzed.
* - Data corrected according to 2.5 µg/cm2 field fortified level average recovery.
a When results were <LOQ, a value of one-half the LOQ was used in the calculations (i.e., 0.0125 $\mu g/cm^2$).

Table 4b. Combined TM and MBC DFRs After Two Applications of Topsin® M WSB at the California Site

Sampling Interval	TM + MBC ($\mu g/cm^2$)			
	Repli. 1	Repli. 2	Repli. 3	Avg.[a]
Pre-Application #1	< LOQ	< LOQ	< LOQ	0.025
Post-Application #1	1.755	1.74	2.10	1.86
Day 1 after App. #1	< LOQ	< LOQ	< LOQ	0.025
Day 3 after App. #1	0.99	0.99	1.14	1.04
Day 5 after App. #1	0.59	0.97	0.95	0.84
Pre-Application #2	0.08	0.09	0.1	0.09
Post-Application #2	2.8	2.24	2.37	2.47
Day 1 after App. #2	0.040	0.025	0.025	0.030
Day 3 after App. #2	0.025	0.040	0.025	0.030
Day 5 after App. #2	< LOQ	< LOQ	< LOQ	0.025
Day 7 after App. #2	< LOQ	< LOQ	< LOQ	0.025
Day 14 after App. #2	NA	NA	NA	NA
Day 21 after App. #2	NA	NA	NA	NA
Day 28 after App. #2	NA	NA	NA	NA
Day 42 after App. #2	NA	NA	NA	NA
Day 56 after App. #2	NA	NA	NA	NA
Day 70 after App. #2	NA	NA	NA	NA
Day 84 after App. #2	NA	NA	NA	NA

NA - Samples not analyzed.

a When results were reported as <LOQ, a value of one-half the LOQ (i.e., 0.0125 $\mu g/cm^2$) was used in the calculation.

Versar re-analyzed the data sets using the Microsoft EXCEL 97® linear regression function, on the corrected combined TM and MBC residues for each site separately and then again after averaging the results from the two sites together. For the NC site, the regression analysis was run considering Day 0 to Day 5 data after the second application and a dissipation half-life value of 1.08 days ($R^2 = 0.89$) was calculated. For the CA site, the regression analysis was run using Day 0 to Day 6 (pre-application #2) data after the first application because of the rain events which occurred the day of the second application. Day 1 after the first application results were considered to be outliers and Versar opted to drop these replicates from the regression analysis. A dissipation half-life of 1.78 days ($R^2 = 0.76$) was calculated. Table 5, below, presents the averaged residue levels from both the NC site (after the second application) and the CA site (after the first application). The regression analysis of the two sites averaged together resulted in a dissipation half-life of 1.42 days ($R^2 = 0.90$). Table 6, below, provides a summary of the half-life values.

Table 5. Combined TM and MBC DFRs Averaging Both North Carolina and California Sites[a]

Sampling Interval	TM + MBC ($\mu g/cm^2$)			
	Repli. 1	Repli. 2	Repli. 3	Avg.[a]
Post-Application	2.42	2.46	2.48	2.45
Day 1 after App.	1.51[b]	1.38[b]	2.23[b]	1.71
Day 3 after App.	0.60	0.59	0.70	0.63
Day 5 after App.	0.34	0.57	0.59	0.50
Day 7 after App.	0.053[c]	0.058[c]	0.063[c]	0.058

a Averaged data from NC site collected after the second application with data from the CA site collected after the first application.
b These values only represent the NC site because this sampling interval was dropped from the CA data set.
c One-half the LOQ values used for NC site data because all values were <LOQ.

Table 6. Calculated Half-lives

Source	Regression Scheme	Site	Half-life (days)	R^2
Author	TM only	NC	0.94	---
Author	TM only	CA	1.53	---
Versar	TM and MBC combined	NC	1.08	0.89
Versar	TM and MBC combined	CA	1.78	0.76
Versar	TM and MBC combined	Both sites averaged	1.42	0.90

Data Variability

Versar examined data variability as part of the linear regression exercise. Coefficients of variance (CVs) ranged from 5.4 percent to 44.6 percent for the combined TM/MBC residues at the NC site and 8.33 percent to 25.6 percent at the CA site. The variability of the data after the two sites were averaged together decreased to a range of 1.25 to 27.8 percent. There are no specific requirements concerning the variability of replicate samples in the Pesticide Assessment Guidelines.

Compliance Checklist

Compliance with OPPTS Series 875, Occupational and Residential Exposure Test Guidelines, Group B: Postapplication Exposure Monitoring Test Guidelines, 875.2100, Transferable Residue Dissipation, is critical. The itemized checklist below describes compliance with the major technical aspects of OPPTS 875.2100, and is based on the "Checklist for Residue Dissipation Data" used for study review by the U.S. EPA/OPP/HED. Additional data gaps identified in the study (not covered by the checklist) are also presented below:

- *Typical end use product of the active ingredient used.* This criterion was met.

- *Site(s) treated representative of reasonable worst-case climatic conditions expected in intended use areas.* This criterion was partially met. Although California and North Carolina sites represent significant climate variations found in strawberry production farming, EPA generally requires testing at a minimum of three sites.

- *End use product applied by application method recommended for the crop. Application rate given and should be at the least dilution and highest, label permitted, application rate.* These criteria were mostly met. The application rate used was the maximum application rate per application as specified on the label for Topsin® M WSB. However, the maximum application rate per season was not met (2.8 lb a.i./A per yr). This would have involved 4 applications at 0.7 lb a.i./A per application. The minimum application volume for strawberries was not specified, a GPA of 30 was used.

- *Applications occurred at time of season that the end-use product is normally applied to achieve intended pest control.* This criterion was met.

- *If multiple applications are made, the minimum allowable interval between applications should be used.* This criterion was mostly met. Two applications were scheduled to be made 7 days apart at both sites, however the second application at the NC site took place 8 days after the first application due to a rain event.

- *Meteorological conditions including temperature, wind speed, daily rainfall, and humidity provided for the duration of the study.* This criterion was met, however, the source of the weather data collected at the CA site was not provided.

- *Reported residue dissipation data in conjunction with toxicity data must be sufficient to support the determination of a reentry interval.* This criterion was partially met. No toxicity data were provided.

- *Residue storage stability, method efficiency (residue recovery), and limit of quantitation (LOQ) provided.* These criteria were met. Storage stability was discussed using the field fortified recovery results. A separate storage stability study was not done. Field fortified and laboratory fortified recovery values were provided in the report. The LOQ was 0.025 $\mu g/cm^2$ for both TM and MBC. The LOQ and the LOD were used synonymously.

- *Duplicate foliar and/or soil samples collected at each collection period.* This criterion was satisfied. Triplicate samples were collected at each sampling interval.

- *Control and baseline foliar or soil samples collected.* The criterion was met. Control samples were collected from the control plot at five different sampling intervals. No soil samples were collected.

- *Sufficient collection times to establish dissipation curve.* This criterion was met. Samples were collected until Day 84 after the second application. However, TM and MBC residue levels declined rapidly due to rain events occurring close to or else on the application dates. The NC site had minimal rain after the second application and the residue levels dropped below LOQ by Day 7 after the second application. Increased sampling intervals just after the applications (i.e., hourly and daily) may have provided a more accurate regression analysis.

Pertinent data gaps and other issues critical to the scientific validity and regulatory acceptability (i.e., Subdivision K compliance) of the study, not already addressed, are presented below. The following issues were identified:

- The reproducibility of replicate samples collected from NC at the same time interval was poor. The coefficient of variance for replicate samples at the NC site ranged from 5.4 percent to 44.6 percent for TM/MBC combined.

- OPPTS 875.2100 (an Update to Subdivision K) specifically requires that DFR data be collected from at least three geographically distinct locations for each formulation. In this study, DFR samples were collected from two locations.

- On page 15 of the Study Report (5.1 Data used for kinetics calculations), the author reported that "only the data following the first application at the NC site and data following the second application at the CA site were used." The sites should be reversed in this statement. The rain event which caused the problems occurred on the day of the second application at the CA site and not the NC site. The author also stated that the data from the first day of sampling after the second application was anomalous at the CA site but it was data from the first day of sampling after the first application that was anomalous.

- The analytical method did not appear to be validated prior to the initiation of the study nor the initiation of the field sample analyses.The protocol stated that the collected field samples were to be dislodged within the EPA recommended 4 hours after collection but this was never confirmed in the field phase summary.

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PC Code:	**102001**
HED File Code	**12000 Exposure Reviews**
Memo Date:	**09/28/2001**
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